Recombinant Antibody Directed Against Human Sperm Antigen

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Field of the Invention

The present invention is directed to compositions and methods for contraception. In particular, the invention is directed to recombinant monoclonal antibodies against sperm surface proteins, and methods for immunocontraception utilizing such monoclonal antibodies.

Background of the Invention

Development of new contraceptives is necessary to provide accessible birth control to all individuals regardless of sociological, financial, or educational limitations. This is especially relevant as the Food and Drug Administration has requested that all of the spermicidal formulations marketed in the US undergo clinical testing and may recommend removing existing detergent based spermicidals from the marketplace if a new spermicidal is introduced. These evaluations have been deemed necessary as the active ingredient in all US marketed spermicides, the non-ionic detergent Nonoxynol-9, has been correlated with a higher incidence of urogenital infections and cervicovaginal inflammation in women repeatedly using this method of birth control [Gupta et al. *J. of Infectious Diseases*, (1998) **178(2)**: 446-450, McGroarty et al., (1994) *J. of Urology*, **152(3)**: 831-833]. In addition recent studies have linked Nonoxynol-9 use with an increased risk of contracting HIV.

As an alternative to chemical detergents, the use of monoclonal antibodies as safe active agents for topical spermicides has been suggested [Cone et al., (1994) *Am. J. Reprod. Immunol.* **32**: 114-131]. A wide variety of monoclonal antibodies have been studied as potential sperm-reacting agents. Among those studied is the anti-human sperm monoclonal antibody S19, described in US Patent

No. 5,830,472 the specification of which is expressly incorporated herein. This antibody was obtained by immunization of mice with human sperm homogenates. S19 recognizes a tissue-specific epitope on its cognate antigen Sperm Agglutination Antigen-1 (SAGA-1), a highly acidic glycoprotein found over the entire surface of ejaculated human sperm [Diekman et al., (1997) Biol. of Reproduction, **57(5)**: 1136-1144; Diekman et al., (1999) FASEB J., August 1999].

Direct evidence for surface localization of the SAGA-1 antigen was provided by immunofluorescence of viable human spermatozoa and electron microscopic immunolocalization [Homyk and Herr, (1993)]. Both light and electron microscopy immunolocalization demonstrate SAGA-1 on the entire sperm plasmalemma. This localization of the epitope is optimal for allowing the S19 monoclonal antibody to function at a variety of levels to impede sperm progress in the female tract. The S19 IgG_1 mAb exhibits important biological actions *in vitro* including agglutination and immobilization of spermatozoa, inhibition of tight binding between human sperm and the zona pellucida and blockage of sperm penetration of cervical mucus. [Anderson et al, J. Reprod. Immunol., 10:231-257 (1987), Cone, *Am. J. Reprod. Immunol.* **32:** 114-131, Mahoney et al, J. Reprod. Immunol., 19:269-285 (1991).]

The strong agglutination of sperm by this antibody has been demonstrated visually on videotape [Diekman et al., (1997) Biol. of Reproduction, **57(5)**: 1136-1144]. In this experiment, within ten minutes after the addition of S19 ascites to live motile human sperm, spermatozoa were tightly bound in agglutination floccules, and no free-swimming spermatozoa were seen after thirty minutes. In other experiments using higher titers of antibody, agglutination occurred within 30 seconds after addition of S19 ascites to live motile human sperm. The agglutinating activity of S19 when the antibody is used in conjunction with a delivery vehicle was investigated using Novasome® liposomes that are specifically formulated for antigen or antibody delivery. Novavax, Inc. (Rockville, MD) provided our laboratory with a Novasome® formulation that contained native S19 monoclonal antibody molecules bound to the surface of positively-charged liposomes. This S19-Novasome® formulation was tested using the sperm agglutination assay. S19-Novasomes® agglutinated sperm at the same efficacy as a 1:20 dilution of the S19 ascites. These results indicate that the

S19 monoclonal antibody can have the same effect on sperm function when incorporated into a commercially available delivery system.

Isojima et al. (1990) have reported that S19 mAb inhibits the sperm binding activity of another monoclonal antibody (mAb), H6-3C4. The H6-3C4 mAb was generated through the fusion of mouse myeloma cells with lymphocytes from an infertile woman. Isojima and colleagues concluded that the H6-3C4 mAb represents an anti-sperm activity responsible for the patient's infertility. The H6-3C4 mAb agglutinates human sperm, immobilizes sperm and reacts with the entire sperm surface, a pattern similar to that of the S19 mAb. Based on studies that utilized immunohistochemistry and absorption with powdered tissues, [Isojima et al. (1990), Wiley-Liss, New York, pp.359-376] concluded that the H6-3C4 cognate antigen(s) is a seminal plasma component that coats the sperm surface. S19 and H6-3C4 epitopes have been shown to lie on identical molecules [Diekman et al., (1999) FASEB J., August 1999] implicating SAGA-1 as a factor in clinical antibody-mediated infertility.

Detailed purification schemes and analyses were carried out to identify the S19 antigen [Diekman et al., (1999) FASEB J., August 1999; CIP to US Patent #5,830,472 (1998)]. Microsequence analysis of affinity-purified SAGA-1 by Edman degradation showed that the first seven amino acids were GQNDTSQ (SEQ ID NO: 18), the eighth was undetermined (designated X), and the ninth, tenth, and eleventh amino acids were designated as SSP. This sequence is homologous to the twelve amino acid peptide core of the mature CD52 glycoprotein. A commercially-available CAMPATH-1M antibody that reacts with the C-terminal tripeptide of CD52 and its GPI-anchor was used to demonstrate that the S19 and CAMPATH-1M antibodies identify an identical series of bands on 1-D blots ranging from 15-25 kD as well as nearly identical patterns of immunoreactive spots (15-25kD, pI 2.5-3.0) on 2-D blots. Immunofluorescent analysis using both antibodies on human sperm showed the same pattern of fluorescence over the entire sperm surface. These assays demonstrate that the SAGA-1 and CD52 glycoproteins are highly homologous. However, through a series of Western blot analyses, it was demonstrated that the S19 antibody did not recognize the lymphocyte CD52 antigen that was recognized by CAMPATH-1M [Diekman et al., (1997) Biol. of Reproduction, 57(5): 1136-1144; Diekman et al., (1999) FASEB J., August 1999; CIP to US Patent #5,830,472 (1998)]. These results

indicate that SAGA-1 and CD52 have carbohydrate structures that are immunologically distinct. SAGA-1 and CD52, therefore, are glycoforms, i.e. glycoproteins with the same core peptide but with differing carbohydrate structures. Analysis of multiple tissues by Western blotting and immunohistological techniques further demonstrated that the S19 carbohydrate epitope is expressed specifically in the epididymis (see US Patent #5,830,472). Furthermore, it has been determined that while the S19 and H6-3C4 antibodies both react with the glycoprotein SAGA-1, the two monoclonal antibodies bind to two separate and distinct carbohydrate epitopes.

The ability of the S19 mAb to inhibit the penetration of bovine cervical mucus was examined using the TruTrax assay (Humagen, Charlottesville, VA) following the manufacturer's instructions with modification. Mouse ascites containing the S19 mAb was mixed with bovine cervical mucus reconstituted from lyophilized powder. The S19-cervical mucus mixture was injected into the TruTrax capillary chamber. An aliquot of human semen was placed at the end of the capillary chamber, spermatozoa were allowed to penetrate the cervical mucus, and their progress was observed by light microscopy. In regions of high sperm density, spermatozoa were agglutinated in same manner observed with the slide agglutination assay. Therefore the S19 mAb is considered to be an excellent candidate for the development of an antibody-based, intravaginal spermicide based on its ability to inhibit sperm function *in vitro*.

The use of the S19 mAb as an antibody-based, intravaginal spermicide suffers form the disadvantage that the MHS-8 hybridoma is currently the only source of native S19 antibody. Large scale antibody production either in tissue culture or mouse ascites is not cost effective for a spermicidal product. Thus there is a need for an expression system for generating the S19 antibody as a means for providing an inexpensive source for spermicide development.

Summary of the Invention

The present invention is directed to a recombinant antibody (RASA) that binds to a human sperm surface antigen, Sperm Agglutination Antigen-1 (SAGA-1), and the use of such antibodies in diagnostic and contraceptive applications. It is anticipated that RASA will be utilized in a number of applications including 1) the

active ingredient of a spermistatic agent, 2) a component of a spermicidal contraceptive, and 3) as a reagent to purify and/or detect human spermatozoa.

Brief Description of the Drawings

Fig. 1 is a schematic diagram of the linking and cloning reaction used to prepare RASA.

Fig. 2 is a representation of an SDS-PAGE immunoblot of a purified preparation of SAGA-1bound by RASA.

Fig. 3 is a representation of an SDS-PAGE immunoblot of bacterial periplasmic fraction preparations (lanes 1, 3 amido stain) bound by anti-E Tag Ab (lanes 2, 4), demonstrating the presence of RASA as a monomer and as multimers. This was demonstrated by adding bacterial periplasmic fractions containing RASA to either reducing (Fig. 3 left panel) or non-reducing (Fig. 3 right panel) running buffer.

Detailed Description of the Invention

In describing and claiming the invention, the following terminology will be used in accordance with the definitions set forth below.

As used herein, "nucleic acid," "DNA," and similar terms also include nucleic acid analogs, i.e. analogs having other than a phosphodiester backbone. For example, the so-called "peptide nucleic acids," which are known in the art and have peptide bonds instead of phosphodiester bonds in the backbone, are considered within the scope of the present invention.

As used herein, the term "purified" means that the molecule or compound is substantially free of contaminants normally associated with the molecule or compound in a native or natural environment.

As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water and emulsions such as an oil/water or water/oil emulsion, and various types of wetting agents.

As used herein, the term "antigen-binding region" refers to the variable region of a monoclonal antibody that directly participates in recognition and binding to an antibody's corresponding antigen. The antigen binding region includes at least

two peptide domains, the light variable chain and the heavy variable chain (i.e. a Fv fragment). The antigen-binding region may include a linker that binds the two peptide domains to one another.

As used herein, the term "linker" refers to three or more molecules that are covalently bound to one another and serve as a bridge to covalently bind one compound/polymer to a second compound/polymer.

As used herein, the term "liposome" refers to a generally spherical or spheroidal cluster or aggregate of amphipathic compounds, including lipid compounds, typically in the form of one or more concentric layers, for example, monolayers or bilayers. The liposomes may be formulated, for example, from ionic lipids and/or non-ionic lipids.

As used herein, "effective amount" means an amount sufficient to produce a selected effect. For example, a contraceptive effective amount of an anti-sperm antibody is an amount sufficient to reduces fertility by at least 70% which is an effectiveness that is unattainable with current detergent based spermacides.

As used herein an anti-sperm antibody is an antibody that is specific for sperm cells and promotes at least one of the following activities: agglutination and/or immobilization of spermatozoa, inhibition of tight binding between human sperm and egg vestments, including the cumulus oophorus, the zona pellucida and the oolemma, or blockage of sperm penetration of cervical mucus.

As used herein a spermicidal agent is an agent that reduces a sperm's ability to fertilize an egg without necessarily killing the sperm.

As used herein, the term "biologically active fragments" of the S19 antibody encompasses natural or synthetic portions of the full-length monoclonal antibody that are capable of specific binding to the SAGA-1 antigen.

The term "peptide" encompasses a sequence of 3 or more amino acids wherein the amino acids are naturally occurring or synthetic (non-naturally occurring) amino acids. Peptide mimetics include peptides having one or more of the following modifications:

1. peptides wherein one or more of the peptidyl --C(O)NR-- linkages (bonds) have been replaced by a non-peptidyl linkage such as a --CH_2-carbamate linkage

(--$CH_2OC(O)NR$--), a phosphonate linkage, a -CH_2-sulfonamide (-CH_2--$S(O)_2NR$--) linkage, a urea (--NHC(O)NH--) linkage, a --CH_2 -secondary amine linkage, or with an alkylated peptidyl linkage (--C(O)NR--) wherein R is C_1-C_4 alkyl;

2. peptides wherein the N-terminus is derivatized to a --NRR_1 group, to a --NRC(O)R group, to a --NRC(O)OR group, to a --$NRS(O)_2R$ group, to a --NHC(O)NHR group where R and R_1 are hydrogen or C_1-C_4 alkyl with the proviso that R and R_1 are not both hydrogen;

3. peptides wherein the C terminus is derivatized to --$C(O)R_2$ where R_2 is selected from the group consisting of C_1-C_4 alkoxy, and --NR_3R_4 where R_3 and R_4 are independently selected from the group consisting of hydrogen and C_1-C_4 alkyl.

Naturally occurring amino acid residues in peptides are abbreviated as recommended by the IUPAC-IUB Biochemical Nomenclature Commission as follows: Phenylalanine is Phe or F; Leucine is Leu or L; Isoleucine is Ile or I; Methionine is Met or M; Norleucine is Nle; Valine is Val or V; Serine is Ser or S; Proline is Pro or P; Threonine is Thr or T; Alanine is Ala or A; Tyrosine is Tyr or Y; Histidine is His or H; Glutamine is Gln or Q; Asparagine is Asp or N; Lysine is Lys or K; Aspartic Acid is Asp or D; Glutamic Acid is Glu or E; Cysteine is Cys or C; Tryptophan is Trp or W; Arginine is Arg or R; Glycine is Gly or G, and X is any amino acid. Other naturally occurring amino acids include, by way of example, 4-hydroxyproline, 5-hydroxylysine, and the like.

Synthetic or non-naturally occurring amino acids refer to amino acids which do not naturally occur *in vivo* but which, nevertheless, can be incorporated into the peptide structures described herein. The resulting "synthetic peptide" may contain amino acids other than the 20 naturally occurring, genetically encoded amino acids at one, two, or more positions of the peptides. For instance, naphthylalanine can be substituted for trytophan to facilitate synthesis. Other synthetic amino acids that can be substituted into peptides include L-hydroxypropyl, L-3,4-dihydroxyphenylalanyl, alpha-amino acids such as L-alpha-hydroxylysyl and D-alpha-methylalanyl, L-alpha.-methylalanyl, beta-amino acids, and isoquinolyl. D amino acids and non-naturally occurring synthetic amino acids can also be incorporated into the peptides. Other derivatives include replacement of the naturally occurring side chains of the 20 genetically encoded amino acids (or any L or D amino acid) with other side chains.

As used herein, the term "conservative amino acid substitution" are defined herein as exchanges within one of the following five groups:

I. Small aliphatic, nonpolar or slightly polar residues:
Ala, Ser, Thr, Pro, Gly;

II. Polar, negatively charged residues and their amides:
Asp, Asn, Glu, Gln;

III. Polar, positively charged residues:
His, Arg, Lys;

IV. Large, aliphatic, nonpolar residues:
Met Leu, Ile, Val, Cys

V. Large, aromatic residues:
Phe, Tyr, Trp

This invention also encompasses nucleic acid molecules characterized by changes in non-coding regions that do not alter the phenotype of the polypeptide produced therefrom when compared to the nucleic acid molecule of the present invention. As used herein, the term "nucleic acid" encompasses RNA as well as single and double-stranded DNA and cDNA.

The present invention is directed to recombinant forms of anti-sperm antibodies and the use of such antibodies as spermistatic/spermicidal agents. One preferred embodiment of the present invention is directed to a recombinant derivative of the anti-sperm monoclonal antibody S19 (deposited, under Budapest Treaty conditions, at the ATCC, on Jun. 26, 1996, Deposit No. HB12144). In particular the invention is directed to recombinant miniantibodies of S19. As used herein the term miniantibody includes fragments of the S19 antibody including but not limited to the Fv fragment (a heterodimer consisting of V_H and V_L), single-chain Fv fragments (wherein the V_H and V_L domains are covalently linked) and the Fab fragment (consisting of the complete light chain with the domains V_L and C_L as well as the first two domains of the heavy chains V_H and C_{H1}).

Antibody fragments of small size (i.e. miniantibodies) are of particular advantage in many applications. In diagnostic applications (e.g. ELISA, RIA, etc.),

the smaller molecule's surface decreases the problems of nonspecific interactions, which are known to frequently involve the constant domains. The same is true in using antibody fragments as ligands in affinity chromatography. The smaller molecular dimensions of miniantibodies also enhances the ability of the injected antibody to penetrate tissues and reach its intended target (Colcher et al., 1990, J. Natl. Cancer Inst. 82, 1191-1197). Furthermore, expression yields and secretion efficiency of recombinant proteins are also a function of chain size (Skerra & Pluckthun, 1991, Protein Eng. 4, 971) and smaller proteins are preferred for this reason. Recombinant antibodies have been developed which are expressed in bacteria (Ward, et al., 1989, Nature 341, 544-546 and Huse, et al., 1989 Science 246, 1275-1281, each of which is incorporated herein by reference.) or on the surface of filamentous bacteriophage (Barbas, et al., 1992, Proc Natl Acad Sci USA 89, 10164-8; Burton, et al., 1991, Proc Natl Acad Sci USA 88, 10134-7; Barbas, et al., 1992, Proc Natl Acad Sci USA 89, 4457-61; and Barbas, et al., 1991, Proc Natl Acad Sci USA 88, 7978-82, each of which is incorporated herein by reference).

In accordance with one embodiment of the present invention, an antigen binding molecule is provided that contains a Fv-fragment of a monoclonal antibody directed against the human sperm surface antigen, SAGA-1, wherein the binding molecule does not use the constant antibody domains of the monoclonal antibody. In particular, in accordance with one embodiment a recombinant derivative of the S19 monoclonal antibody is provided that includes only the biologically active fragments of antibody S19 and not any of the other peptide domains of the native S19 antibody. The S19 recombinant derivatives of the present invention have at least 70% to 75% of the original S19 antibody protein sequence deleted and yet retain their specificity for the S19 antigen.

Fv fragments have a tendency to dissociate into their component V_H and V_L domains. Therefore, it is advantageous to link the two domains covalently. The two domains can be covalently linked together using standard linkers known to those skilled in the art. Preferably the linker comprises a chain of at least three molecules and the two domains are linked either in the orientation V_H -linker-V_L or V_L -linker-V_H. The resulting fragments are called single-chain Fv fragments. One

particular way of linking them is by designing a peptide linker between them (Bird et al.,1988, Science 242, 423; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85, 5879).

The present invention provides a recombinant antibody capable of specifically binding to SAGA-1 wherein the recombinant antibody comprises only two peptide fragments of the S19 antibody and the fragments are joined together by a linker. The isolated S19 peptide fragments, or peptide mimetics thereof, in accordance with the present invention constitute less than 50% of the S19 native peptide sequences and more preferably represent less than 30% of the native S19 protein sequences, yet the miniantibody retains its ability to bind specifically to the S19 antigen.

The S19 peptide fragments used to form the S19 miniantibody of the present invention comprise the variable domains of the S19 heavy and light chains. In one embodiment the two peptide fragments have amino acid sequences of

a) SEQ ID NO: 1 and SEQ ID NO: 3, respectively;

b) fragments of SEQ ID NO: 1 and SEQ ID NO: 3, respectively;

c) peptide mimetics of SEQ ID NO: 1 and SEQ ID NO: 3, respectively;

or

d) amino acid sequences identical to SEQ ID NO: 1 and SEQ ID NO: 3 but having 1 to 5 conservative amino acid substitutions in each of SEQ ID NO: 1 and SEQ ID NO: 3, respectively. In accordance with one embodiment, the recombinant S19 miniantibody comprises a single chain Fv fragment, wherein the single chain Fv fragment consists of two biologically active fragments of the S19 monoclonal antibody and a linker that covalently binds those two fragments to one another. In one embodiment the first of the two biologically active fragments comprises a 20 consecutive amino acid sequence identical in sequence to a 20 consecutive amino acid sequence of SEQ ID NO: 1, and the second of the two biologically active fragments comprises a 20 consecutive amino acid sequence identical in sequence to a 20 consecutive amino acid sequence of SEQ ID NO: 3.

In an alternative embodiment the recombinant S19 miniantibody comprises a single chain Fv fragment, wherein the single chain Fv fragment consists of SEQ ID NO: 1, SEQ ID NO: 3 (or amino acid sequences identical to SEQ ID NO: 1 and SEQ ID NO: 3, but having 1 to 3 conservative amino acid substitutions in each

of SEQ ID NO: 1 and SEQ ID NO: 3) and a linker that covalently binds SEQ ID NO: 1 to SEQ ID NO: 3.

The linker moiety can be selected from a broad range of compounds that are capable of covalently linking two peptides (or peptide mimetics). The linker moiety is typically a polymer and one preferred polymer linker is a peptide. The peptide linker is typically 5-30 amino acids in length, and one preferred peptide linker is a $(Gly_4Ser)_n$ polymer. One preferred linker is a peptide of 10-20 amino acids in length, more preferably 12-17 amino acids. In one embodiment a 15 amino acid long peptide is used as the linker, for example a $(Gly_4Ser)_3$ linker having the sequence of SEQ ID NO: 5. In accordance with one embodiment, the recombinant antibody comprises a sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 11 and SEQ ID NO: 15.

The recombinant antibodies of the present invention can be further modified using standard techniques to include signal peptides or peptide tags that assist in the production and purification of the synthesized products. In addition the antibodies can be coupled to various diagnostic labels, including radioisotopes and flourescent markers. In one embodiment the antibody is linked to a bioactive agent that is capable of exerting a biological effect *in vitro* and/or *in vivo*. Bioactive agents include, for example, toxins, virucides and microbicides, therapeutic agents, pharmaceutical agents, drugs, synthetic organic molecules, proteins, peptides, oligosaccharides, steroids, steroid analogs, chelators or metal binding peptides, enzymes, peptides bearing a detectable label (e.g. a radioisotope or fluorophore) and genetic material, including nucleic acids. In one embodiment the recombinant antibody is coupled to an adenylate cyclase toxin or other toxin, like ricin.

In accordance with one aspect of the invention the recombinant form of the anti-sperm antibody, S19, is used as a spermistatic agent. Inhibition of both sperm motility and gamete interactions by the S19 mAb demonstrated that this antibody blocks sperm function at multiple steps in the process of fertilization and indicate S19 is an excellent candidate for a topical contraceptive based on an anti-sperm antibody. Therefore, the S19 miniantibody can be used as the sole active agent in a spermistatic gel or cream for contraceptive use. In another embodiment, the S19 miniantibody is

combined with, or coupled to, an effector molecule such as a toxin, virucide or microbicide to create a spermicidal agent.

To provide an effective spermicidal/spermistatic gel or cream, the recombinant antibody must be provided in an effective vehicle. Suitable delivery vehicles are described in Cone et al., 1994, *Am. J. Reprod. Immunol.* **32:** 114-131 and Alexander, 1995, *Scientific Amer.* September: 136-141 and are known to those skilled in the art. Applicants have developed a particular vehicle for the presentation of recombinant S19 in agglutinating form, by using the commercially available liposome delivery system (Novasome®) available from Novavax, Inc. of Rockville, Md. These liposomes are specifically formulated for antigen or antibody delivery. Novasomes® having recombinant S19 antibody molecules bound to the surface of these non-phospholipid positively charged liposomes function effectively as a spermicide/spermistatic in a spermicide/spermistatic gel, cream or foam.

The Novasome® formulation has been tested using the native S19 monoclonal antibody and the sperm agglutination assay. At a 1:10 dilution the S19-Novasome® vehicle agglutinated sperm at the same efficiency as a 1:20 dilution of the S19 ascites fluid. These results indicate that the S19 monoclonal antibody has the same effect on sperm function when incorporated into commercially available delivery systems, as in native ascites fluid.

Alternative delivery systems are available to those of skill in the art. Prominent among these are lipid-conjugated peptides, see e.g., Deres et al, Nature, 342:561-564 (1989) and ISCOMs, see e.g., Takahashi, Nature, 344:873-875 (1990). Other preparations, including hydrophobic emulsions and saponins, have been developed in the past for the processing and presentation of specific peptides, and may be used in connection with the antibodies of the spermicide/spermistatic addressed herein. See, e.g., Raychaudhuri et al, Proceedings of the National Academy of Science, U.S.A. 89:8308-8312 (1992) and Newman et al, J. Immunol. 148:2357-2362 (1992). Other vehicles, including the presentation of the antibodies on a membrane, such as that expressed by recombinant virus (i.e., recombinant viral schemes wherein the DNA encoding the antibody is expressed in an recombinant cell together with a structural membrane protein) may be used.

Of the available varieties, in addition to the non-phospholipid liposomes described above, ISCOMs are commonly used in vaccines for the presentation of antigens and they offer desirable features. ISCOMs form cage-like membrane structures into which or on which the antibody can be presented. ISCOMs have previously been used in connection with the presentation of antigens, but they similarly present antibody proteins in an exposed, virus-like structure. In this respect, other vehicles known for the presentation of active proteins, including co-polymer spheres, and virus-like particles (VLPs) have been known to achieve results similar to the immune-stimulating complexes, or ISCOMs. Alternatively, the attachment of the antibody, via coupling agent, to the surface of a microsphere, may be suitably used in conjunction with acceptable manufacturing techniques for the formulation of gels and creams consistent with this approach.

Thus, in accordance with one embodiment of the present invention a composition and method is provided for inhibiting fertilization. The method comprises the step of administering a composition comprising the anti-SAGA-1/CD52 antibody to a female in an amount effective to interfere with fertilization. The essence of the this contraceptive composition is the incorporation, in a suitable carrier, of a sufficient concentration of the S19 miniantibody in an appropriate vehicle to effectively inhibit (agglutinate or bind) all sperm present in an ejaculate.

In accordance with one embodiment a composition is provided comprising a recombinant antibody and a pharmaceutically acceptable carrier, wherein the recombinant antibody is a derivative of monoclonal antibody S19, wherein at least 75% of the protein sequence has been deleted and the recombinant antibody specifically binds to the S19 antigen. The composition comprises an effective amount of the recombinant antibody to allow the composition to function as a contraceptive. For example, the composition can comprise a concentration of recombinant monoclonal antibodies sufficient such that one dose of said composition effectively binds 98-100, more preferably 99 or 100, percent of all sperm cells present in an average ejaculate sample. The typical ejaculate contains sperm densities in a range from 80-120 million/mL. Ejaculates range from 3 to 7 ml, thus the composition should contain sufficient antibodies to effectively bind about 240 to 840 million sperm.

In accordance with one embodiment, a formulation comprising the S19 miniantibody and a delivery vehicle is applied to the vagina for binding and immobilizing spermatozoa and thus function as a contraceptive. In one embodiment the delivery vehicle is a liposome and the recombinant monoclonal antibodies are present on the surface of liposome. Preferably the liposomes are non-phospholipid positively charged liposomes and in one embodiment the liposomes are Novasomes®.

A temporary method of inhibiting fertilization is also provided in accordance with one embodiment of the invention. The method comprises administering to an individual preformed anti-sperm antibodies from another individual of the same or different species. This is known as passive immunity. One example of such immunity is the protection afforded to a fetus and newborn by placental transfer of maternal antibodies, as well as the transfer of antibodies through milk. Another example is the pooled adult gamma globulin frequently used to prevent or modify the effects of exposure to measles, chicken pox, hepatitis, smallpox and tetanus. These acquired antibodies, however, are gradually utilized by interaction with the antigen or catabolized by the body, and thus the protection is eventually lost.

In one embodiment, a passive immunity composition is used for contraception, wherein the composition comprises the S19 miniantibody of the present invention as an active agent. In particular, the composition comprises an amount of S19 miniantibody sufficient to provide circulating titers of the antibody, in a patient requiring the same, in an amount sufficient to transude to the secretions of the reproductive tract and inhibit the ability of sperm to fertilize an egg. This inhibitory effect includes inhibition of one or more of the following: sperm maturation, sperm passage and gamete interactions (including binding of sperm to the egg and sperm penetration). The passive immunity composition can be administered to either a male or female to decrease the fertility of that individual, and can be administered by any of the standard routes used to administer pharmaceutical agents including oral, topical and parenteral routes. Preferably the passive immunity composition is administered intravenously. Accordingly, the present invention also incorporates a method of promoting contraception in a mammal through passive immunity. The method comprises administering a composition comprising an amount

of S19 miniantibody sufficient to provide circulating titers of the antibody in a sufficient amount to inhibit the ability of sperm to fertilize an egg.

The recombinant antibodies of the present invention can also be used in the broad field of diagnostic and therapeutical medicine. For example, the S19 miniantibody has utility as a reagent to purify sperm cells from other cell types, and for evaluating human semen samples. Regarding diagnostic applications, the properties of proteins bound by the S19 mAb, including the SAGA-1/CDS52 protein, make two different types of diagnostic testing possible. Initially, when SAGA-1 or variants thereof are used as a contraceptive vaccine, it is necessary to monitor the development of antibodies to the SAGA-1 protein or variants thereof. Certain potential candidates may lack a suitable immune response system, and many individuals vary in the antibody titer generated in response to any immunogen, or protocol of immunogen administration. Development of an adequate antibody titer may be easily confirmed using the SAGA-1 protein as an antigen standard. Using the agglutination assay described above with respect to the S19 miniantibody, one can easily determine the concentration necessary for 100 percent agglutination. This concentration of SAGA-1 protein, properly immobilized and presented, is tested against a sample drawn from the patient. Achievement of 100 percent binding suggests an adequate antibody titer. Because any such vaccination program must be conducted under clinical supervision, the testing format may be conventional, using ELISA, Western blotting analysis or other established art formats.

There is, in addition, increasing interest in simple-to-use, "user friendly" commercial diagnostics, that is, over the counter diagnostics. The reasons for interests in this type of diagnostic, and the variety of uses for such a diagnostic, are discussed in detail in U.S. Patent No. 5,605,803, which is incorporated herein by reference. The diagnostics disclosed therein employ the SP-10 binding antibody, MHS-10, or antibodies having the binding characteristics of those expressed by a hybridoma cell line at the ATCC available under Accession No. HB 10039. As described in the incorporated application, this type of over the counter diagnostic finds application in forensic environments, can be useful in determining the presence of normal germ cell lineage, finds substantial value in those experiencing difficulty in achieving conception, in vasectomized males and the testing of vasectomized males,

both by males and females, as well as the testing of vasovasostomized males, to determine the success of surgical reconnection. For any of a variety of reasons, determination of the presence of sperm in a biological sample, either an ejaculate or a sample derived from an ejaculate or from the male reproductive tract, is of significant medical importance. The antigen bound by the S19 miniantibody, the SAGA-1 protein and corresponding variants and recombinant derivatives, having the S19 CDR-responsive epitope, can be employed in assay formats such as those described in the incorporated U.S. Patent No. 5,605,803.

Thus, the S19 antibody may be bound to a solid phase and used to capture the SAGA-1 protein, and detect, isolate and quantitate the presence of sperm in a sample. As noted earlier, the S19 miniantibody advantageously does not contain the constant domains of the native S19 antibody and thus the smaller molecule's surface decreases the problems of nonspecific interactions, which are known to frequently involve the constant domains. Recognition of the presence of the SAGA-1 antigen (or sperm) may be completed by use of a second monoclonal or polyclonal reagent. See, e.g., Shen et al, Am. J. Reprod. Immunol., 29:231-240 (1993).

Other assay formats could utilize a wick or dipstick, and antibody coded colored beads. In all these variations, the essential reagent is the S19 miniantibody, and any sperm or SAGA-1 protein present in the sample tested will be bound or captured by the immobilized antibody. Thus, in accordance with one embodiment a composition, comprising the recombinant S19 miniantibody immobilized on a solid support is provided as a diagnostic device. In one preferred embodiment the antibody is covalently bound to the solid support. The solid support can be either in the form of a monolithic structure or in particulate form.

Additionally, the S19 miniantibody may be utilized to purify and/or detect human sperm. This methodology could find application in the fields of fertility counseling, forensic diagnoses in the case of rape, or evaluation of the effectiveness of vasectomy or vasovasostomy. Thus one aspect of the invention is directed to a method of detecting or isolating sperm from a biological sample. In one embodiment the method comprises contacting the sample with a composition comprising the recombinant S19 miniantibody immobilized on a solid support under conditions that allow the binding of SAGA-1 to the S19 miniantibody, washing the solid support to

remove unbound and non-specific bound material, and detecting the presence of sperm. In one embodiment the S19 antibody is covalently linked to a solid matrix in particulate form (for example a magnetic particle or agarose bead) that are used to form an affinity column. The immobilized S19 miniantibody compositions can also be used to isolate sperm cell DNA from a sample containing multiple cell types, in sufficient purity to allow PCR based analysis of the sperm DNA, and identification of the source individual with a high degree of certainty. The method comprises the steps of contacting the sample with a solid support, having recombinant S19 miniantibody immobilized thereon, for a time sufficient to allow sperm cells present in the sample to bind to the S19 miniantibody, washing the binding substrate to remove unbound and non-specific bound material, lysing the cells that remain bound to the antibody; and purifying the cellular DNA.

The present invention also encompasses multimeric forms of the recombinant antibody (i.e. dimers, trimers, etc.) of the single chain Fv fragments. Initially, it was expected that the S19 miniantibodies would be unable to cross-link sperm alone, since cross-linking requires the antibody bridging two antigen binding sites. Accordingly, in one embodiment the recombinant antibody is further modified to include additional peptide sequences that allow the formation of bi- or multifunctional antibody-fragment fusion proteins and miniantibodies. For example each of the recombinant antibodies can be provided with an antigenic peptide sequence whereby the use of a secondary antibody to that epitope results in a bivalent recombinant antibody. However, such modification may not be necessary, for as shown in Fig. 3 RASA can be produced as a monomer, dimer, trimer, and higher weight multimers (Fig. 3 shows Western blotted bacterial periplasmic fractions probed with anti-E Tag antibody). Presumably, the multimer forms result from the light chain domains of one single chain Fv fragment interacting with the heavy chain of a second single chain Fv fragment. This multimerization enables RASA to agglutinate spermatozoa on its own. One embodiment of the present invention is directed to a multimer recombinant antibody comprising at least two single chain Fv fragments of the S19 antibody in a covalent or noncovalent interaction, wherein the orientation of the monomeric fusion proteins in the dimer is identical with respect to their N- and C-termini. In another embodiment a multimer recombinant antibody

composition is prepared that consists only of the recombinant RASA antibody.

The present invention also encompasses nucleic acids that encode the described S19 recombinant miniantibodies. In one embodiment a nucleic acid sequence is provided that encodes for one or more of the variable domains of the S19 monoclonal antibody. Preferably, the nucleic acid comprises sequences that encode for a single chain Fv fragment. In accordance with one embodiment, a nucleic acid sequence is provided comprising a single chain Fv fragment that consists of SEQ ID NO: 2, a nucleic acid linker; and SEQ ID NO: 4, wherein said linker is covalently bound to SEQ ID NO: 2 and SEQ ID NO: 4. The nucleic acid linker is ligated to the DNA sequences encoding SEQ ID NO: 2 and SEQ ID NO: 4 in frame, so the expression of the nucleic acid sequence produces a functional Fv fragment. In accordance with one embodiment the nucleic acid sequence linker has the sequence of SEQ ID NO: 13, and the single chain Fv fragment has the sequence of SEQ ID NO: 16.

In creating RASA the majority of the murine antibody sequence (80% of the total mouse IgG) has been removed which lessens the likelihood of generating human anti-mouse antibody (HAMA) responses in patients using RASA-containing spermicides/spermistatics. In accordance with one embodiment the murine antibody variable region framework codons are exchanged for human antibody variable region framework codons (see SEQ ID NO: 11) while preserving the CDR sequences to "humanize" the RASA and thus further lessen the likelihood of generating human anti-mouse antibody (HAMA) responses in patients using RASA-containing spermicides/spermistatics. In addition, the native mouse Fv fragment sequences can be modified, using standard techniques known to those skilled in the art, to make the gene more suitable for expression in prokaryotic cells. For example one or more codons of the gene encoding the mouse Fv fragments can be exchanged for codon sequences more commonly used by bacteria. It is anticipated that the removal of infrequent bacterial codons will allow the miniantibody to be expressed in *E. coli* at higher concentrations. In one embodiment of the invention a nucleic acid sequence is provided wherein the nucleic acid comprises a single chain Fv fragment selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 16, SEQ ID NO: 17.

These cloned single chain Fv fragments can be inserted into commercially available DNA vectors (expression vectors) to express the encoded gene protein product. These expression vectors have promoter sequences and other regulatory sequences necessary for expression in host cells. The technique of using expression vectors to introduce exogenous genes and express their protein products in a host cell is well known to those familiar with the art. For example, the expression vector pET21a is commercially available and can be used to express proteins in *E. coli*. Alternatively the protein can be expressed in a eukaryotic cell, such as yeast, using Pichia expression vectors (i.e. pHIL-D2) commercially available from Invitrogen. The Baculovirus system is also commercially available and can be used to express the single chain Fv fragment genes in insect cultures.

Once the single chain Fv fragment gene or fragment thereof has been subcloned into an expression vector, the resulting vector can be used to transform a host cell, using procedures known to those familiar with the art. Such transformation procedures include but are not limited to microinjection, mircoprojectile bombardment, electroporation, calcium chloride premeablization, polyethylene glycol permeabilization, protoplast fusion or bacterial mediated mechanisms such as *Agrobacterium tumafaciens* or *Agrobacterium rhizogenes*.

Host cells may be selected from any cell in which expression of modified proteins can be made compatible, including bacteria, fungus, yeast, plant cells and animal cells. Suitable host cells include prokaryotes selected from the genus Escherichia and eukaryotes selected from the genus Pichia. The transformed host cells will synthesize the modified protein which can be isolated and purified using standard methods known to those familiar with the art. One embodiment of the present invention comprises a method for using a host cell transformant, having heterologous DNA sequences encoding a single chain Fv fragment protein, to produce a recombinant miniantibody that specifically binds to the SAGA-1 antigen. The method comprises the step of culturing the cell transformant under conditions conducive to the expression of the recombinant antibody protein. In accordance with one embodiment a single chain Fv fragment having the sequence of SEQ ID NO: 10 is inserted into a bacterial expression vector, a suitable bacterial host (such as *E. coli*) is

transformed with the vector, and the transformant is used to express the single chain Fv fragment.

The invention also relates to a process for preparing the single chain fragments of the present invention, characterized in that the genes coding for the Fv-fragment, and, if desired, the linking peptide are cloned into an expression plasmid, a host cell is transformed with said expression plasmid and cultivated in a nutrient solution, and the monomeric fusion protein is expressed in the cell or secreted into the medium.

Example 1

Cloning of the S19 miniantibody

In order to make a more cost-effective reagent, the S19 variable region cDNA (the nucleic acid sequences encoding the native S19 antigen binding regions) was cloned into a bacterial expression vector and expressed as a recombinant S19 miniantibody (called RASA). Over the last few years, methods have been described (Skerra and Pluckthun, 1988, Science 240, 1038-1040; Better et al., 1988, Science 240, 1041-1043) that allow functional antibody fragments to be produced in *Escherichia coli*.

To identify the native S19 antibody chain sequences, MHS-8 hybridoma RNA was extracted, reverse transcribed, amplified, and sequenced. MHS-8 hybridoma cells secreting S19, an IgG1 antibody, were processed for RNA collection utilizing the FastTrack 2.0 kit (Invitrogen). The variable regions of the antibody that create the SAGA-1 binding site were identified using oligonucleotides specific for IgG1 heavy chain and light chain variable region subunits. Reverse transcription (RT) and polymerase chain reaction (PCR) amplification of S19 hybridoma RNA were performed in a single reaction using the Access RT-PCR system from Promega. Amplified products were analyzed on an agarose gel, and the bands isolated. Cloning of the bands was performed using the pCR-Script SK(+) system (Stratagene).

Positive colonies of each clone, heavy or light chain, were chosen and grown up for plasmid purification. Sequencing of the positive clones was performed in the University of Virginia Biomolecular Research Facility using an ABI Prism 377

Automated DNA Four Color Sequencer. By comparing the deduced clone sequences to known murine IgG1 sequences it was found that the clones represented the appropriate murine antibody chains.

To reduce the potential for human-anti-mouse antibody (HAMA) responses, approximately 80% of the native S19 sequence was removed when making the recombinant antibody. The nucleotide sequence of the heavy chain (SEQ ID NO: 4) and the light chain (SEQ ID NO: 2) were used to construct specific heavy or light chain primers. The single-stranded cDNA made from S19 hybridoma (this cell line is deposited as ATCC Deposit No. HB12144) RNA amplified by PCR (Access RT-PCR kit, Promega, Madison, WI). The resulting heavy (354 base pairs (bp)) and light chain (348bp) fragments were gel-purified to remove unincorporated primers and any extraneous amplification products. These fragments were sequenced in the University of Virginia Biomolecular Research Facility using an ABI Prism 377 Automated DNA Sequencer and compared to the original sequences determined above.

Importantly, in the re-sequencing it was found that there were two errors in the original light chain sequence presented in US Patent No. 5,830,472. The first error was a two base pair addition introduced by the original primers used for RT-PCR which would have caused a frameshift in the expressed sequence. The second change was due to a sequence misread by the automated sequencer coding for "RR" in the original sequence in CDR1 that is actually "HS" (at amino acids 31 and 32 of SEQ ID NO: 1).

Each cDNA was quantitated to ensure that equivalent amounts of both cDNAs were added to the subsequent assembly reaction. The purified heavy and light chain products were assembled into a single cDNA expression construct using 100 nucleotide (nt) overlapping cDNA linker primers (SEQ ID NO: 6 and SEQ ID NO: 7). The linker fragments annealed to the 3' end of the heavy chain cDNA and the 5' end of the light chain cDNA (Fig. 1) to produce a single-chain Fv (scFv) sequence where the heavy and light chains are joined by a cDNA encoding a $(Gly_4Ser)_3$ flexible linker sequence. This linker maintains the correct reading frame and properly aligns the chains to create the correct SAGA-1 binding site. The resulting ScFv is 753bp long.

To prepare the ScFv for insertion into the pCANTAB 5E vector (Recombinant Phage Antibody System (RPAS), Pharmacia Biotech, Uppsala,

Sweden); the cDNA was PCR amplified with primers to introduce restriction sites for cloning. SfiI and NotI sites were added to the 5' and 3' ends of the linked fragment, respectively. The resulting ScFv cDNAs were gel purified, quantitated, and sequentially digested with SfiI and NotI to generate cohesive ends for insertion into the pCANTAB expression vector previously cut with these enzymes.

The S19 ScFv fragment was ligated into pCANTAB and transformed into *E. coli* HB2151 cells to produce soluble recombinant antibodies. The insertion site in plasmid pCANTAB incorporates a 39bp nucleotide sequence (13 amino acid "GAPVPYPDPLEPR"; SEQ ID NO: 14) called the E Tag at the 3' end of the ScFv (SEQ ID NO: 9 and SEQ ID NO: 8 represent the entire nucleotide and protein sequences, respectively, with the E Tag). Antibodies to this peptide tag can be used to identify full-length recombinant proteins. Plasmids were transformed into the HB2151 cell line (Pharmacia Biotech, Uppsala, Sweden). Transformed cells were grown on plates containing glucose and ampicillin to select for colonies containing the ScFv insert.

Depending on the clone, soluble antibodies may be secreted into the culture supernatant, localized to the bacterial periplasm, and/or inside the bacterial cell cytoplasm. To determine the localization of the recombinant antibodies the culture supernatant, periplasmic extract, and whole cell extracts were examined by Western blot analysis. Individual colonies were picked and grown in 5ml LB cultures overnight at 37°C. These cultures were then added to 500ml of fresh media containing ampicillin and grown for 3 hours at 37°C. Cultures were equilibrated to 30°C for 30min., IPTG added to 1mM, and incubated for an additional 3 hours at 30°C. Cell pellets were collected by centrifugation and supernatants saved. To generate periplasmic fractions, the pellets were resuspended in 5ml of chloroform, incubated at room temperature for 15 minutes, 25ml of 10mM Tris-HCl pH8 was added and the sample was centrifuged to remove cellular debris. The resulting supernatant contains proteins from the periplasmic fraction. Whole cell fractions were made by resuspending pellets in 10mM Tris-HCl pH 8. The protein extracts were separated by SDS-PAGE (Laemmli, (1970) *Nature* **227:** 680-685), transferred to nitrocellulose (Towbin et al., (1979) *PNAS* **76:** 4350-4354), blocked with nonfat milk in PBS, and incubated with anti-E Tag antibodies. Immunoreactivity of the anti-E

Tag antibody with recombinant antibodies on blots was identified by the development of dark bands using tetramethylbenzidine (TMB) reagent (KPL Labs). Only recombinant proteins found in the periplasmic fractions of clones showed strong activity with the anti-E Tag antibody and were the expected 29kD molecular mass. The E-tagged recombinant proteins were not identified in cultures of constructs without inserts, or in uninduced cultures. Periplasmic fractions containing the E-tagged recombinant antibodies were used for further experiments.

Example 2

Identification of an active recombinant miniantibody to the human sperm surface.

Each E-tagged recombinant protein was tested for binding to the SAGA-1 human sperm antigen through Western blot analysis. Ejaculated human sperm were solubilized, microcentrifuged to remove genomic DNA and cellular debris, separated by SDS-PAGE, and transferred to nitrocellulose. Blots were incubated with recombinant antibodies followed by incubation with the anti-E Tag secondary antibody. Native S19 mAb and anti-E Tag antibody alone served as positive and negative controls, respectively. Immunoreactivity with SAGA-1 on immunoblots was identified by the development of dark blue bands using TMB. Both the original S19 mAb and one recombinant clone, RASA (recombinant anti-sperm antibody), identified an identical set of bands ranging from 15-25 kD indicating that the RASA clone retained proper confirmation to react with SAGA-1.

Reactivity of the S19 recombinant miniantibody with the surface of human sperm was investigated using immunofluorescence as performed by Homyk and Herr (1993, *J. Reprod. Immunol.* **22:** 237-256) for the initial characterization of the S19 mAb. Human sperm ejaculates were washed twice in Ham's F-10 medium and centrifuged. Viable spermatozoa were incubated with RASA. The native S19 mAb and anti-E Tag antibody alone were included as positive and negative controls, respectively. Spermatozoa were incubated with FITC-conjugated antibody, and washed twice. RASA showed localization over the entire human sperm surface by immunofluorescent analysis, identical to that observed with the native S19 antibody.

This finding visually confirmed the recombinant miniantibody bound the entire human sperm surface.

Agglutination of human spermatozoa by RASA was investigated with the *in vitro* slide agglutination test as described by Diekman et al., 1997, *Biol. of Reproduction.* **57(5):** 1136-1144. Viable human spermatozoa were mixed with an equal volume of RASA. S19 and spermatozoa alone were included as positive and negative controls, respectively. Each sample was placed on a microscope slide, covered with a cover slip, incubated for 10 minutes, and examined for extent and pattern of agglutination. The agglutination of spermatozoa was observed as the cross-linking, or clumping together, of spermatozoa indicating that RASA binds spermatozoa. Both the RASA antibody alone or a combination of RASA and the anti-E Tag antibody, when added to a human sperm solution, resulted in agglutination of the sperm. The control, anti-E Tag antibody in combination with human sperm, produced no obvious agglutination.

As ScFvs, such as RASA, have only one antigen binding site, it was not expected that the recombinant could cross-link sperm alone, as cross-linking requires the antibody to have bi- or multi valency. For this reason, an anti-E Tag antibody was used as a cross-linking agent in one of the agglutination slides. However, as shown with other ScFvs [Arndt, KM et al., (1998) *Biochemistry* **37(37):** 12918-26.], further studies indicated that RASA can be produced as a monomer, dimer, trimer, and higher multimers. This multimerization enables RASA to agglutinate spermatozoa on its own. This was demonstrated by adding bacterial periplasmic fractions containing RASA to either reducing (Fig. 3 left panel) or non-reducing (Fig. 3 right panel) loading buffer. Samples were run on 15% SDS-PAGE gels, Western blotted with anti-E Tag antibody, and developed with TMB. As seen in the non-reduced fraction, the RASA exists as not only a 29kD monomer, but also as a ~60kD dimer, ~90kD trimer, and a variety of higher weight multimers.

Example 3

Use of the S19 miniantibody as a diagnostic agent

The presence of the SAGA-1 over the entire human sperm surface, and the creation of RASA, make this antibody/antigen system useful for several diagnostic

applications. The reasons for interests in this type of diagnostic, and the variety of uses for such a diagnostic are discussed in detail in US Patent #5,605,803, the disclosure of which is expressly incorporated herein. The diagnostics therein employ the SP-10 binding antibody, MHS-10, or other antibodies having the binding characteristics of those expressed by a hybridoma cell line at the ATCC available under Accession No. HB10039. A diagnostic of this type finds application in forensic environments, in assisted reproduction, in testing of vasectomized males, as well as the testing of vasovasostomized males to determine the success of surgical reconnection. Determination of the presence of human semen in a biological sample, either an ejaculate, a sample derived from an ejaculate, or from the male reproductive tract, is of significant medical importance. RASA is also useful for the isolation of human sperm for subsequent analyses or procedures such as PCR for forensic applications, or in isolating sperm in cases of oligospermia for subsequent assisted reproduction.

In accordance with one embodiment the recombinant antibody of the present invention can be linked to solid particulate matter, magnetic beads, ligands or the like that allow for the separation of the antibodies from a solution after the antibody binds to the target SAGA-1 antigen. Such methods can be utilized to isolate or concentrate live sperm cells for use in fertilization. Alternatively the methods can be used to detect or purify small amounts of sperm for use in forensics. The recombinant antibody can also be used as a positive control for monitoring the serum of individuals for antibodies to sperm surface antigens. Finally the recombinant antibodies of the present invention can be labeled with a fluorescent or visible marker and used to quantitate sperm in a sample.